and what is expected from its ranking in the Hofmeister series. Multi-parameter smFRET experiments on the α-helix under varying salt concentrations were carried out to gain a more detailed insight into the induced conformations.
Addition of KCl allowed the peptide to adopt an α-helical conformation whereas GndCl denatured by swelling the chain. NaClO4 at 4M condenses the chain into a rapidly fluctuating collapsed state. With the help of CD, MD and spFRET we could show that two denaturing ions (Gnd, ClO4-) work in two very different mechanisms.

220-Pos Board B6
Quantifying the Effects of Ion Condensation on the Conformations of Molecular Type I Collagen
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The polyampholytic nature of type I molecular collagen continues to exhibit a rich and complex molecular behavior. It has been known for some time that, due to the charge structure of the molecule, ions condense on the collagen triple helix. This ion condensation has been shown to exhibit preferential binding of certain ions. in this work we have shown that not only does preferential ionic binding occur but that the condensation of the ions on the molecule causes drastic conformational changes.
Bovine dermal type I collagen molecules are suspended in solutions with varying ionic species and concentration. The conformations of the molecules are measured by imaging with AFM as deposited onto mica substrates. It is found that with all ionic species tested, increasing the ionic concentration corresponds with a straightening of the molecule, calculated as a persistence length using the worm-like chain model.
The complex behavior of the collagen molecule appears in the comparison of the rates of persistence length increase with ionic concentration. At the low salt concentrations used here, 0.001-0.1M, this rate of increase presents as a power law. The salt species chosen for this work aimed to elucidate the effects of mono- versus divalent salts and changing the anion or cation species.
The dependence of persistence length on ionic species correlates with the degree of ionic condensation reported in previous results. Now, not only do we know that ionic condensation occurs, but it also plays a significant role in the conformations of the molecules.

221-Pos Board B7
Salt Effects on the Conformational Stability of Small Peptides
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Experimental and computational work has shown that the presence of ions in solution affects the conformations of proteins. The driving force behind the effect of ions on shifts in the conformational equilibria of proteins is not known. We believe that ions modulate a peptide's hydration, thereby causing it to undergo a conformational shift. We have tested this hypothesis by performing molecular dynamics simulations on a peptide with the primary structure AEAAAEA in different salts at different concentrations to examine ion effects on the electrostatic repulsion between side chains, ion-side chain binding, as well as peptide hydration. These are three factors that may drive the shifts in peptides' conformational equilibria as influenced by ions. Salts included in this study were NaCl and KCl at concentrations of 0.5, 1.0 and 2.0 M. Simulation trajectories were analyzed with radial distribution functions, preferential interaction formalism, and various energy distributions. Free energy calculations for the entire conformational space of this peptide in each saline environment were calculated using metadynamics simulations.

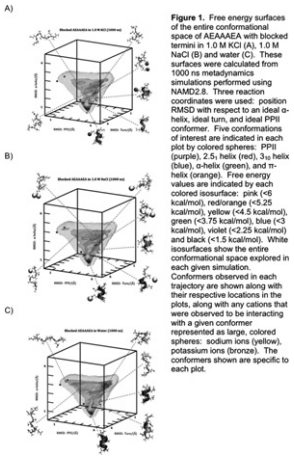

Figure 1. Free energy surfaces of the entire conformational space of AEAAAEA with blocked termini in 1.0 M KCl (A), 1.0 M NaCl (B) and water (C). These surfaces were calculated from 1000 ns metadynamics simulations performed using NAMD2.8. Three reaction coordinates were used: position RMSD with respect to an ideal α-helix, ideal turn, and ideal PPII conformer. Five conformations of interest are indicated in each plot by colored spheres: PPII (purple), 2.5_1 helix (red), 3_{10} helix (blue), α-helix (green), and π-helix (orange). Free energy values are indicated by each colored isosurface: pink (<6 kcal/mol), red/orange (<5.25 kcal/mol), yellow (<4.5 kcal/mol), green (<3.75 kcal/mol), blue (<3 kcal/mol), violet (<2.25 kcal/mol) and black (<1.5 kcal/mol). White isosurfaces show the entire conformational space explored in each given simulation. Conformers observed in each trajectory are shown along with their respective locations in the plots, along with any cations that were observed to be interacting with a given conformer represented as large, colored spheres: sodium ions (yellow), potassium ions (bronze). The conformers shown are specific to each plot.

222-Pos Board B8
Amyloid Beta Misfolds and Increases its Affinity for Cell Membranes at the Initial Step of Aggregation
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An amyloid protein changes its molecular conformation as it aggregates. However, this aspect of aggregation has remained difficult to measure. Alzheimer's amyloid beta (Aβ) monomers are thought to be relatively unstructured, while the mature aggregates have a β-sheet-rich hairpin-like conformation where the two termini come close together. It is not clear at what stage of aggregation this change ('misfolding') takes place, and how this affects the properties of the aggregate, such as its stability, shape and toxicity. It is also not known why monomers, presumably present in healthy humans, are non-toxic, and how toxicity arises at some stage of aggregation. Here we use Fluorescence Correlation Spectroscopy, Forster Resonance Energy Transfer (FRET), fluorescence quenching, and vesicle and cell binding assays to address these questions in physiological buffer conditions. We show that the major conformational transition takes place right at the first step of aggregation, viz. with the formation of the small oligomers. This change predominantly involves the core region of the peptide, as the structures of the two termini remain relatively unchanged between the monomer and the oligomer. In both the species, the N-terminus is almost fully solvent exposed, while the C-terminus is much more shielded. This monomer to oligomer transition increases the affinity of Aβ for artificial lipid vesicles by at least an order of magnitude. Confocal microscopy shows that the oligomers at physiological concentrations have a strong affinity for the plasma membrane of living HEK 293T cells, while the monomers at the same concentration do not have any detectable affinity. Our results imply that the major change of molecular structure occurs at the initial step of aggregation, and suggest that this plays a major role in transforming nontoxic monomers into toxic cell-adherent oligomers.

223-Pos Board B9
Plasma Membrane Topology of the Insulin Receptor: Insights from Computational Modeling
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The insulin receptor in the plasma membrane is composed of two alpha-subunits, each linked to a beta-subunit and to each other by disulfide bonds. It has been proposed that both alpha-subunits are extracellular and anchored to the plasma membrane via a single transmembrane (TM) helix in the N-terminal region of each beta-subunit. However, we find that newer sequence analysis algorithms predict that the alpha-subunits of human insulin receptor (P06213) are largely intracellular and each contains a single TM helix in the N-terminal region. The MEMSAT-SVM method predicts (after correcting for a 19 residue N-terminal signal peptide) that the alpha-subunit contains a TM helix between residues 64 and 79, with a 63 amino acid ectodomain and a large 676 residue cytoplasmic domain. The existence of a large intracellular domain is consistent with the presence of 10 caveolin-binding (CB) motifs (Öx ÖxxxxÖ, ÖxxxxÖxxÖ, where Ö is the aromatic amino acid F, Y, or W), which exist within most caveolin-associated proteins where caveolin is accessible only intracellularly. Our computational analysis of the insulin receptor beta-subunit sequence confirms a single TM helix between residues 194 - 218 (UniProtKB places the TM helix within residues 194-213), with 1 CB motif proximal to the TM helix (W175 - Y182). Similar topologies were obtained with insulin receptor sequences from a variety of species. The insulin receptor co-isolates with lipids and the purified insulin receptor readily forms lipid complexes. Thus, caveolin binding motifs may direct the insulin receptor tetramer to caveolin-rich lipid rafts. The topology of the insulin receptor and CB motifs is relevant to understanding the microdynamics of insulin receptor movement between caveolar and non-caveolar regions of the plasma membrane as well as insulin-mediated events such as endocytosis, membrane hyperpolarization, and regulation of $Na^+/K^+/H^+$ ions.

224-Pos Board B10
Structural Insights into the Regulation and the Recognition of Histone Marks by the Set Domain of NSD1
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Background: The nuclear receptor binding SET domain (NSD) protein is a family of three histone-lysine N-methyltransferase (HMTase), NSD1, NSD2/MMSET/WHSC1, and NSD3/WHSC1L1 that are critical in maintaining the chromatin integrity. NSD1 methylates H3K36 and H4K20 and is associated with acute myeloid leukemia, multiple myeloma, and lung cancer. NSD1 is amplified in multiple myeloma, lung cancer, neuroblastomas and glioblastomas. NSD2 methylates H3K4 and H4K20 and is linked to prostate cancer, multiple myeloma and glioblastoma. NSD2 is found over expressed in fifteen different cancers and is associated with tumor aggressiveness or prognosis in most types of cancers. NSD3 methylates H3K36 and is associated with both lung and breast cancer along with the acute myeloid leukemia. The amplification of either NSD1 or NSD2 triggers the cellular

transformation. Reducing NSDs activity through specific and selective lysine-HMTase inhibitors appears promising to help suppressing cancer growth. In absence of ligand, the histone-binding site of NSD1 is occluded preventing any access to the catalytic groove. Therefore, we hypothesized that the SET domain of NSD1 has specific mechanisms to recognize histone marks unlike other HMTase.
Methods: We used computational methods to investigate the structural mechanisms happening in the SET domain during the binding of the H4-histone tail.
Results: Our finding exposes a key regulatory and a recognition mechanism driven by the flexibility of a loop at the interface of the SET and postSET region who rotates ~45° and translated 7Å opening the SET domain for the binding of the peptide ligand. This regulatory loop acts as a seat belt for the ligand and contributes to the discrimination and the substrate specificity. HMTase inhibitors are scare but our data bring significant insight into the design of specific and selective NSD-HMTase inhibitors.

225-Pos Board B11
High Level Expression of Proopiomelanocortin in *E. coli* Cells using Optimized Codons
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Proopiomelanocortin (POMC), a precursor protein, serves as the source of numerous biologically active peptides, including MSHs, ACTH, CLIP, LPH, and β-endorphin. POMC is processed at pairs of basic amino acid residues in a tissue-specific manner by pro-hormone convertase 1 and 2 (PC1/2). However, little is known concerning its own role and the mechanism associated with its processing, based on its tertiary structure. In addition, little information is available concerning the relation between the tertiary structures of the mature peptides and POMC.
In order to obtain structural information related to the processing mechanism of POMC, recombinant POMC was previously expressed using a T_7-promoter system in *E. coli* cells and the purified POMC was crystallized. However, the contaminating proteins affected the formation of POMC crystals. The expression level of POMC in *E. coli* cells was quite low, which made it difficult to eliminate contaminating proteins. Therefore, a system that permits the expression of much higher levels of the protein is required for the structural analysis of POMC.
For this purpose, the chemically synthesized cDNA encoding POMC, optimized at the codons and GC contents, was used to overcome the codon bias of *E. coli*. The PCR-amplified cDNA of POMC was introduced into several different expression vectors, such as pET17b and pPal7, and expressed in several types of *E. coli* cells. The recombinant POMC was well over-expressed in SHuffle T7 *E. coli* cells and purified by a combination of Ni-affinity and hydroxyapatite chromatography. The results will be discussed in this paper.

226-Pos Board B12
Crystal Structure of *E. coli* Tryptophanase in "Semi-Holo" Form: An Insight into Allostery of the Enzyme
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A new crystal form of *E. coli* tryptophanase (tryptophan indole-lyase, Trpase) (space group $P4_32_12$, a=b=109.97 Å, c=238.40 Å) was obtained under the same conditions as the tetragonal crystals of holo *E. coli*. Trpase. The structure was solved by molecular replacement at 3.2 Å resolution using the coordinates of apo *E. coli* Trpase (PDB code 2OQX) as a search model and refined to R=21.3 %, R_{free}=28.9 %. Out of two polypeptide chains contained in the asymmetric unit, one was found in holo form with PLP covalently attached to Lys271, while the other appeared to be in the apo form. The overall conformation of the holo subunit is the same as in the holo form of tyrosine phenol-lyase. The apo subunit is found in a wide-open conformation very similar to the one observed in the crystal structure of apo Trpase. Taking into account the flexibility of apo Trpase as seen in the known structures and difference in the crystallization conditions (pH, precipitant) and crystal packing, this finding is quite unexpected. We suggest that apo Trpase is found in the solution predominantly in the wide-open conformation which partially closes upon binding of PLP. The closed conformation might correspond to the enzyme state with both cofactor and substrate bound, in a way similar to tyrosine phenol-lyase. In addition, the conformation of the loop 301-310 is different in apo and holo subunits of the new structure suggesting that this conformational change is not induced by the oxidation of Cys298.

227-Pos Board B13
Effect of Crystal Packing on Cro Dimer Conformation
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X-ray crystallography is the primary method in structural biology for providing information about protein conformation. However, artificial packing forces in the crystal lattice select just a snapshot of a protein's conformational ensemble, whereas proteins are flexible and may adopt different conformations in order to function. This raises the question, how accurately do X-ray structures describe the solution state of a protein? To address this critical issue, we have established protocols for performing Molecular Dynamics (MD) simulation in solution as well as the crystalline environment and for using network analysis to study the conformational ensembles[1-3].
As a model system, we consider the λ Cro repressor, whose solved X-ray structures range from a closed to an open global conformation. The fully open form is observed both bound and unbound to DNA. Network analysis and a free energy surface constructed from Replica Exchange MD reveal that closed and semi-open conformations are stable in solution, with a modest barrier separating these two states[3]. Yet the fully open conformation, while accessible, lies higher in free energy, indicating it requires stabilization by DNA or crystal contacts. Since a semi-open state is among the low energy conformations sampled in simulation, we propose that this form may initiate DNA recognition and only minor adjustments are needed to achieve the fully open conformation as observed in the functional complex. Subsequent crystal MD simulations estimated the strength of packing interfaces in the lattice, showing the influence of crystal form and mutation in stabilizing different dimer conformations. Our quantitative results will aid analysis of X-ray data in establishing protein structure-function relationships. [1] Vorontsov, I. I. and Miyashita, O. (2009) *Biophys. J.*, **97**, 2532-2540. [2] Campbell, Z. T., et al. (2010) *Biophys. J.* **99**, 4012-4019. [3] Ahlstrom, L. S. and Miyashita, O., *submitted*.

228-Pos Board B14
Integrating Genomic Information with Molecular Simulation to Understand Protein Complex- and Active Conformation Formation in Two-Component Signal Transduction
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Protein function often requires a protein to form a complex or adopt multiple conformations during its functional cycle. Many of these states are transient or unstable and their full structural characterization remains a daunting experimental task. Here, we demonstrate a multi-disciplinary approach that can predict such structures for the common prokaryotic protein class of two-component signal transduction systems (TCS). TCS enable cells to sense and react to external stimuli. A membrane bound sensor histidine kinase (SK) detects an environmental stimulus and forms a complex with a transcription factor/response regulator (RR) transferring a phosphoryl group to mediate a cellular response. The complex is ruled by transient interactions. Despite decades of experimental studies, only few experimental structures are available: none of them trapped during autophosphorylation and only recently was a complex structure of a SK/RR pair structurally resolved [1]. Concurrently, we predicted this complex structure in high agreement (3.5 RMSD) with the experimental work by combining molecular dynamics and statistical genomic analysis [2,3]. Based on this theoretical work, it is now possible to also predict the structural changes occurring during autophosphorylation. Direct coupling analysis [3] identifies innerprotein pairings formed between the HisKa and ATP-binding domains which are not realized in the (inactive) crystal structure. This information can be used in molecular dynamics simulations to identify an active conformation adopted during autophosphorylation in agreement with biochemical mutagenesis data [4].
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